IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In Re the Application of:	)	Group Art Unit: 1656
	)	
Hutton	)	Confirmation No: 4524
	)	
Serial No.: 10/573,280	)	Examiner: Karen C. Carlson
	)	
Filed: December 21, 2006	)	**DECLARATION OF JOHN HUTTON UNDER 37 CFR § 1.132**
	)	
Atty. File No.: 2848-56-PUS	)	
	)	
For: "USE OF ISLET GLUCOSE-6-PHOSPHATASE RELATED PROTEIN AS A DIAGNOSTIC TOOL AND THERAPEUTIC TARGET FOR AUTOIMMUNE DIABETES"		

CERTIFICATE OF TRANSMISSION

I HEREBY CERTIFY THAT THIS CORRESPONDENCE IS BEING FILED ELECTRONICALLY WITH THE U.S. PATENT AND TRADEMARK OFFICE USING THE ELECTRONIC FILING SYSTEM (EFS-WEB) ON September 23, 2009.

SHERIDAN ROSS P.C.

BY: /Robert D. Traver/

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Dear Sir:

I, Dr. John Hutton declare as follows:

1. I am co-inventor of the above-referenced patent application and have first-hand knowledge of all statements made in this Declaration.

2. This Declaration under 37 CFR §1.132 is being submitted in conjunction with a Response to an Office Action mailed on March 23, 2009, the Response being filed herewith.

3. This Declaration provides evidence that demonstrates autoantibodies against Islet Glucose-6-Phosphatase Related Protein (IGRP) molecule are found in diabetic and prediabetic mammals.

4. Autoantibodies to IGRP

Synthetic cDNAs were constructed that encode polypeptide chains that incorporate only the parts of the IGRP molecule that are exposed in the aqueous phase on the intracellular domain of the molecule (see Fig 1). These parts are joined by flexible linkers that encode "hinge" sequences such as the Fc hinge region of immunoglobulin or short repeats of amino acids with short side chains to ensure maximum flexibility. The sequence is preceded by a synthetic leader sequence that can encode a start codon, signal sequences and additional residues for radiolabeling such as methionine (Met) residues (^{35}S) and protein kinase recognition sequences (^{32}P)

5. Assays

Results of radioimmunoprecipitation assays performed with an IGRP construct based on the schematic in Figure 1B is shown in Figure 2. The construct used incorporated a Kozak sequence and a start Met codon in the leader position and 3 repeats of glycine residues as the hinges. The construct was expressed as a ^{35}S labeled protein in a reticulocyte lysate and incubated overnight in 50µl PBS containing 0.15% Tween and 1% albumin following a standard protocol (Yu L, Gianani R, Eisenbarth GS (1994) *Quantitation of glutamic acid decarboxylase autoantibody levels in prospectively evaluated relatives of patients with type I diabetes. Diabetes* 43: 1229-1233). Radioactivity bound to the immunoglobulin was recovered by Protein A affinity beads and filtration. Samples were derived from a random selection of human sera derived from type 1 diabetic patients (n=47) and age- and gender-matched controls derived from the first degree relatives of type 1 diabetes patients who did not exhibit evidence of diabetic autoimmunity (n=16).

The diabetic group were distinguishable from the controls in showing higher level of immunoprecipitation of the ^{35}S labeled construct ($p<0.02$ Mann Whitney statistics; $P<0.01$ T-test with Welch correction; Figure 2A). A Receiver Operator Curve Analysis (Figure 2B) indicated that the assay had a 7.5% sensitivity at 100% specificity and 10.6% sensitivity at 95% specificity. The area under the curve was 0.70 ($p=0.001$).

6. C) T-cell responses to IGRP

Non-obese diabetic (NOD) mice were immunized with membranes from insect cells over-expressing IGRP. Recall responses in lymphocytes from the draining lymph nodes were analyzed using a library of overlapping 18mers. Eight peptides consistently gave positive responses, only 2 of which overlapped with the previously defined epitopes (Mukherjee R, Wagar D, Stephens TA, Lee-Chan E, Singh B (2005) *Identification of CD4+ T cell-specific epitopes of islet-specific glucose-6-phosphatase catalytic subunit-related protein: a novel beta cell autoantigen in type 1 diabetes. J Immunol* 174: 5306-5315).

A) Analysis of CD4 T cell responses to IGRP in NOD mice (see Figure 3).

PLN cells from newly diabetic NOD mice were cultured for 72h in the presence of either peptide (10μg/ml) alone (bars on left side of each grouping; Direct), peptide + blocking mAb 10-3.6.2; (Oi VT, Jones PP, Goding JW, Herzenberg LA (1978) Properties of monoclonal antibodies to mouse Ig allotypes, H-2, and Ia antigens. *Curr Top Microbiol Immunol* 81: 115-120) ("middle" bars of each grouping; + mAb), or peptide + IgG2a isotype control (bars on right side of each grouping; + cont. mAb). ^{3}H-thymidine was included for the final 18h, and DNA incorporated radiolabel measured by liquid scintillation counting. Porcine insulin (100μg/ml) was used as a positive control. Polyclonal CD4 T cell lines isolated from newly diabetic NOD splenocytes were re-stimulated with irradiated autologous splenocytes and IGRP peptide P8. Three days later they were fused with polyethylene glycol to the BWZ.36 TCR α-/β- thymoma and selected with HAT. Ten days later proliferating (HAT resistant) hybrids were screened for antigen responsiveness by induction of LacZ. Data represents the stimulation index of hybrids incubated with antigen relative to those incubated in the presence of APCs alone.

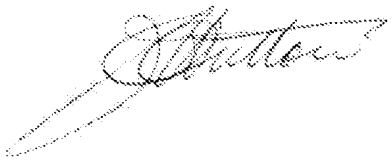
As shown in Figure 3 significant spontaneous responses were observed to 4 peptides (P8, P36, P40, and P41), each of which was selectively blocked by monoclonal 10-3.6.2 (anti-I-A^{k} but cross-reactive with I-A^{g7}). A modest response was observed for P17.

B) Generation of peptide epitope specific T-cell lines reactive to IGRP (see Figure 4). The responses of mouse cell lines derived from new-onset diabetic NOD mice to stimulation with IGRP peptide 8 is illustrated in Figure 4. From 96 wells screened, 10 wells gave a greater than 3-fold stimulation index used as the cut-off for the assay.

7. Conclusion

The results from the analyses in Figure 2 show that human type 1 diabetic subjects do exhibit humoral autoimmunity directed at IGRP. Figures 3 and 4 provide data that incubation of biological samples from diabetic or prediabetic individuals directly with an IGRP peptide (or native or recombinant protein derived from IGRP) provide a diagnostic assay for cell-mediated autoimmunity to this antigen.

8. I hereby declare that all statements made herein of my own are true and that all statements made on information and belief are believed to be true; and further that the statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the subject application or any patent issuing therefrom.



September 23, 2009

Date

John Hutton, Ph.D.

Figure 1 A. Schematic illustration of the model of IGRP orientation in the endoplasmic membrane. **B** is an example of the product of a cDNA that encodes the cytoplasmic loops #2-#8 linked by short flexible linker sequences and similarly connected to a synthetic leader and the native C-terminus of IGRP.

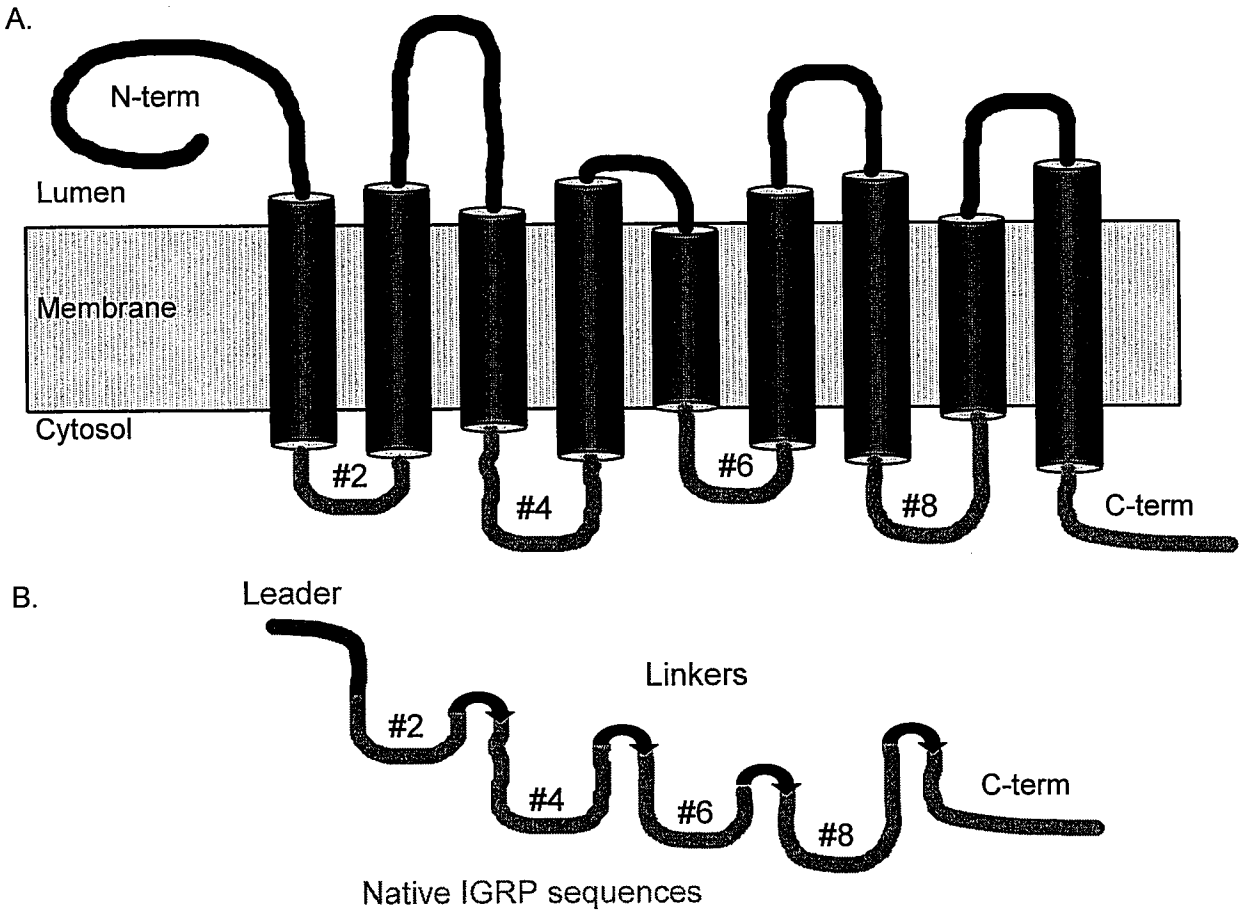


Figure 2: IGRP autoantibody assay results

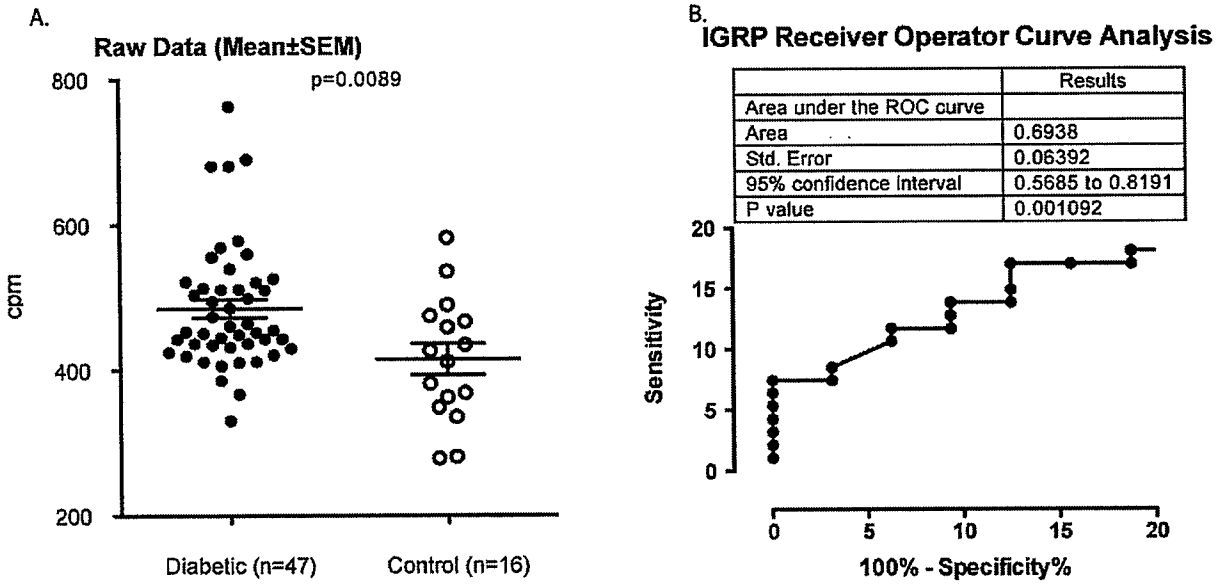


Figure 3. Analysis of CD4 T cell responses to IGRP in NOD mice.

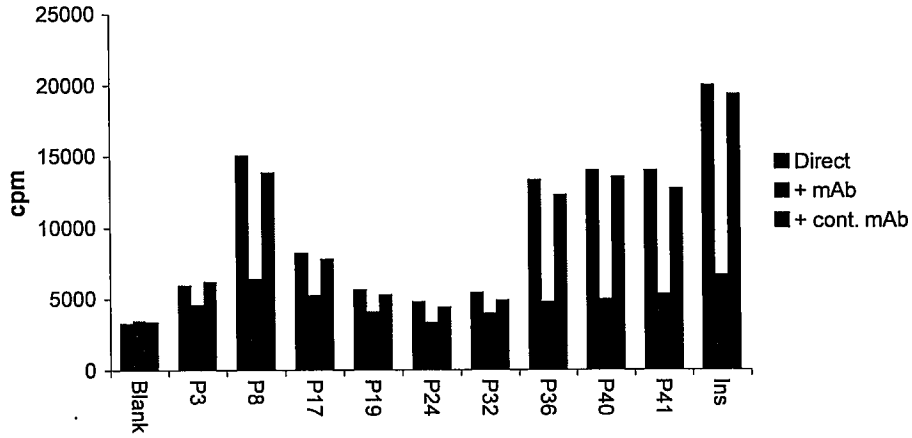


Figure 4 Generation of peptide epitope specific T-cell lines reactive to IGRP.